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DETERMINATION OF SOME MOLECULAR MARKERS ASSOCIATED WITH SUGER CONTENT IN SWEET SORGHUM

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ABSTRACT

The present study was carried out to evaluate three inbred lines of sweet sorghum for their performances to sugar content at the field. These three inbred were chosen as a high sugar content (Wiley) and low sugar content (Roma and Rex) for hybridization to obtain the F1 plants and then selfed to obtain the F2 plants for each cross of Roma X Wiley and Wiley X Rex. These three parental lines and their segregated F2 plans for each cross were evaluated for their performances to sugar content by recording two sugar content – related traits such as sucrose and total soluble solids percentages as well as days to a 50% of heading traits. Bulks of the two extreme F2 plant groups (the highest and the lowest sugar content groups) for each cross and the three parental line plants were tested against seven RAPD, five ISSR and three SSR primers to determine some molecular markers for sugar content in sweet sorghum. However, the three SSR primers were monomorphic, while the others were polymorphic which only five RAPD primers out of them developed molecular markers for sugar content. Five positive markers in the two crosses as well as two positive markers in the cross of Wiley X Rex were detected. On the other hand, three negative markers in the two crosses as well as one negative marker in the cross of Wiley X Rex were also detected.

Keywords: Sweet sorghum, Sugar content, Molecular markers, Marker-assisted selection.

INTRODUCTION

Sweet sorghum is a major cereal for human and animal consumption with a high photosynthesis rate, high biomass yields potential and high percentage of easily fermentable sugars. Sugar content ranged from 9 – 12% of dry matter in sorghum. The frequent occurrences of food shortage in sorghum growing areas and the extension of sorghum cultivation to marginal lands require extensive breeding programs to introduce new varieties fitting small-scale farms needs (Haussmann *et al*., 2000). Compared to maize, sorghum breeding has been neglected in recent decades and the availability of high yielding maize varieties has led to the displacement of sorghum. However, sorghum may bear advantageous genes that are especially useful in conferring resistance to abiotic as well as biotic stresses (Wenzel *et al*., 2001).

Introduction of the polymerase chain reaction (PCR) gave a new boost of the use of DNA markers (Mullis and Faloona, 1987). Michelmore *et al*. (1991) developed the bulked segregant analysis of F_2 plants as a simpler alternative to isogenic line analysis, where the highest and the lowest extreme groups of the F_2 population are bulked for the development of molecular markers associated with a given characteristic. The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of plant breeding program through a number of ways, ranging from fingerprinting of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits and making environmental-neutral selection possible (Ejeta *et al*., 1999). So, molecular markers developed by analysis of randomly amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) have recently shown excellent potential to assist selection of quantitative trait loci (QTLs) associated with economically important traits. In addition, marker-assisted breeding can offer an efficient and rapid means to identify and incorporate adapted germplasm into Egyptian cultivars.

The present investigation aim to test a high parent and two low parents of sweet sorghum in sugar content and their segregated F2 plants which resulted from the hybridization between the high parent

with both of the two low parents with respect to their performances for two sugar content-related traits and to detect some molecular genetic markers associated with sugar content trait via RAPD, ISSR and SSR markers using bulked segregant analysis (BSA) to be used in marker-assisted selection (MAS) programs.

MATERIALS AND METHODS

1. Materials.

The present investigation was divided into three parts; the first part (Field Experiments) was conducted in the Experimental Farm at Faculty of Agric., Al-Azhar Univ., Assiut Branch, the second part (Laboratory analysis) was carried out at Sugar Crops Institute, ARC, Malawy Station Res., and the third part (Molecular genetic analysis) was conducted in the laboratory of Genetics Dept., Fac. of Agric., Ain Shams University.

Three inbred lines of sweet sorghum (*Sorghum bicolor* L.(Moench)) namely; Wiley (high sugar content), Roma and Rex (low sugar content) were chosen for the present investigation. The grains of these three inbred lines were kindly obtained from Sugar Crops Res. Institute, ARC, Giza, Egypt.

2. Methods.

2.1. The evaluated traits analysis.

2.1.1. Field experiment.

These three inbred lines were sown in the field and crossed to obtain the F_1 grains of the two crosses of Roma x Wiley and Wiley x Rex. The F_1 grains of these two crosses were sown in the field and selfed to obtain the F_2 grains. The three parental lines and F_2 grains of the two crosses were grown in a randomized complete block design with three replications during 2008 – 2009 growing season. Each replicate was consisted of 52 ridges for the two crosses. From each cross, three ridges were planted for each parent and 20 ridges for the F_2 plants. Each ridge was four meters long and 60 cm wide. Hills were spaced at 20 cm within ridge and seedlings were thinned at two plants/hill. All cultural practices i.e., irrigation, fertilization and pest control were applied as usual for the ordinary sorghum production.

2.1.2. Traits measurments.

Days from planting to a 50% of heading (flowering) trait for panicle was recorded on a sample of 10 guarded plants in the middle row for each of the three parental lines and all guarded plants in all rows of F_2 plants for each replicate.Total Soluble Solids persentage (TSS%) trait was determined by handle refractometer after extraction of juice. Sucrose percentage trait was determined by the polarization method as described in AOAC (1995). The analysis of variance and multiple comparison were performed according to the method described by Snedecor and Cochran (1968).

2.1.3. Preparation of plant material for molecular analyses.

The F_2 plants represented by 940 and 1090 individual plants for the two crosses of Roma x Wiley and Wiley x Rex, respectively, were classified into groups according to their performances for sugar content-related traits. Leaf samples of the three parental line plants and the two extreme groups of the F_2 individual plants for each cross (the highest four in sugar content and the lowest four in sugar content) were taken for further molecular analyses.

2.2. Molecular marker analysis.

2.2.1. Genomic DNA extraction.

DNeasy™ Plant Mini Kit (Qiagen.Inc., Cat. No. 69104) was used for DNA extraction as described in the manufacturer manual from the three parents and the two extreme groups of the F_2 plants leaf samples for each cross using bulked segregant analysis (BSA) technique.

2.2.2. PCR conditions and electrophoresis.

Seven, five and three primers for RAPD, ISSR and SSR analyses, respectively, were used in this study. Codes and sequences of these primers are listed in Table (1). The amplification conditions and PCR mixture were set according to Williams *et al*. (1990) for RAPD, Sharma *et al*. (1995) for ISSR and Bhattramakki *et al*. (2000) for SSR analysis.

To visualize the PCR products, a 15 ul of each reaction was loaded on 1.2% agrose gels. These gels were run at 90 v for 1 hour, visualized with UV Transilluminator and photographed using UVP gel documentation system (Gel Works 1 D advanced software, UVP).

2.2.3. Data analysis.

Data of polymorphic and monomorphic fragments for each analysis was scored using the UVP gel documentation system. Fragment sizes were estimated using 1-Kb ready load™ DNA ladder as a standard DNA marker (Bioron, Germany).

Table (1): Codes and sequences of the used primers.

Primer type	Primer code	5' Sequence 3'	Annealing temperature
RAPD	A01	5' CAGGCCCTTC 3'	37 ºC
	A03	5' AGTCAGCCAC 3'	
	A14	5' TCTGTGCTGG 3'	
	A16	5' AGCCAGCGAA 3'	
	A20	5' GTTGCGATCC 3'	
	B08	5' GTCCACACGG 3'	
	C20	5' ACTTCGCCAC 3'	
ISSR	HB08	5' (GA) 6 GG 3'	50 ºC
	HB09	5' (GT) 6 GG 3'	
	HB10	5' (GA) 6 CC 3'	
	HB11	5' (GT) 6 CC 3'	
	17898D	5' (CA) 6 GT 3'	
	Xtxp37	F: AACCTAAGAGGCCTATTTAACC	55 ºC
		R: ACGGCGACTATGTAACTCATAG	
	Xtxp84	F: CCGATCAGCACACCAG	50ºC
		R:GTACTAGGTCCAATCCAGC	
	Xtxp115	F: TTGTTTCGGTGACCAC	60ºC
		R: TATCTTTAAATTGCCTTTGTT	

RESULTS AND DISCUSSION

Sugar content-related traits.

1.1. Responses of the three parents.

The means of two sugar content-related traits (Sucrose % and TSS %) and days to a 50% of heading for the high parent (Wiley) and the low parents (Roma and Rex) are shown in Table (2).

Table (2): Means of flowering, sucrose% and TSS% traits for the three tested parents.

Genotypes	Days to a 50% of heading	Sucrose %	TSS%
Roma	**85.00**	**10.12**	**14.60**
Wiley	**93.00**	**12.75**	**19.00**
Rex	**73.00**	**11.50**	**17.50**
Mean	**83.67**	**11.46**	**17.03**
LSD 5%	**3.46**	**0.72**	**0.60**

Days to a 50% of heading trait indicated that Wiley was the late parent (93 days), followed by Roma the moderate parent (85 days) while, Rex was the early parent (73 days). Sucrose percentage trait exhibited marked decrease in the mean values for Roma and Rex parents (10.12 and 11.5%, respectively) comparing with Wiley parent (12.75%) for this trait. Also, TSS percentage trait of Wiley parent showed higher mean value (19%) comparing with Roma and Rex parents (14.6 and 17.5%, respectively) for this trait. These results indicated that there were clear differences among the three tested parents for the three studied traits.

1.2. Responses of the F_2 plants.

The F_2 plants were arranged in descending order according to their frequency in each trait. So, plants with a high frequency in group one were taken to represent the highest F_2 plants, while plants in the

last group were taken to represent the lowest F_2 plants in sugar content in each cross.

According to these classifications, four F_2 plants were chosen to represent the highest F_2 plants and four were chosen as the lowest ones to sugar content of each trait as shown in Table (3) for Roma x Wiley cross and in Table (4) for Wiley x Rex cross. These sixteen plants were used for bulked segregant analysis to develop molecular (RAPD, ISSR and SSRs) markers associated with sugar content in sorghum.

Many authors evaluated two contrasting parents and their segregated F_2 population plants to detect some molecular markers associated with abiotic and biotic stresses as well as yield component and quality traits in these plants. However, their results reflected significant differences between parental genotypes for the studied trait(s) which indicating the variability existed between these parents. Moreover, they classified the segregated F_2 population plants to the highest and the lowest groups based on the studied trait(s) to develop molecular markers using bulked segregant analysis. In this respect, Abdel-Tawab *et al.* (2004) studied total biomass trait in sweet sorghum, Abdel-Bary *et al.* (2005) tested some salt tolerance-related traits in maize, Rashed *et al.* (2006) evaluated some salt tolerance-related traits in sorghum, Atta *et al.* (2006) recorded some iron deficiency-related traits in maize, Fahmy *et al.* (2007) screened some drought tolerance-related traits in rice and Younis *et al. (2007)* measured some salt tolerance-related traits in grain sorghum.

Table (3): The highest and the lowest F_2 plants in sugar content according to two sugar content-related traits and days to a 50% of heading trait in the Roma x Wiley cross.

Group	Days to 50% heading	Sucrose%	TSS%
	The highest groups		
1	85.0	19.3	27.5
2	83.0	16.5	23.0
3	81.0	16.6	22.7
4	80.0	16.3	22.4
Mean	82.25	17.18	23.9
	The lowest groups		
1	105.0	10.0	14.8
2	104.0	9.8	14.6
3	96.0	9.5	14.5
4	90.0	6.8	10.4
Mean	98.75	9.03	13.58

Table (4): The highest and the lowest F_2 plants in sugar content according to two sugar content-related traits and days to a 50% of heading trait in the Wiley x Rex cross.

Plant numbers	Days to a 50% of heading	Sucrose%	TSS%
	The highest groups		
1	73.0	18.1	25.0
2	71.0	16.1	22.3
3	70.0	15.3	21.3
4	68.0	15.0	21.0
Mean	70.5	16.13	22.4
	The lowest groups		
1	94.0	10.2	15.5
2	92.0	10.1	14.9
3	90.0	10.0	14.6
4	87.0	5.0	10.1
Mean	90.75	8.83	13.80

2. Molecular genetic markers for sugar content.

DNA isolated from the three parents (Wiley as the high parent, Roma and Rex as the low parents in sugar content) and DNA bulks of the highest and lowest groups of F_2 segregating population from each cross were tested against seven RAPD, five ISSR and three SSR primers as shown in Figures (1, 2 and 3). The SSR primers were monomorphic, while the others were polymorphic which only five RAPD primers out of them developed molecular markers for sugar content as shown in Table (5).

A03, A16 and A20 primers exhibited seven positive molecular markers for sugar content. However, A03 and A20 primers showed two markers with fragment sizes of 385 and 435 bp, respectively, which were detected in the cross of Wiley x Rex and found in the high parent (Wiley) and the highest F_2 bulk, while they were absent in the low parent (Rex) and the lowest F_2 bulk. Moreover, five markers with fragment sizes of 1059 (A16 primer), 1538, 1293, 372 and 266 bp (A20 primer) were detected in the two crosses and found only in the high parent (Wiley) and the highest F2 bulk for each cross, while they were absent in the two parents (Roma and Rex) and the lowest F_2 bulk for each cross.

On the other hand, A01 and B08 primers exhibited four negative molecular markers for sugar content. However, B08 primer showed a marker with a fragment size of 288 bp which was detected in the cross of Wiley x Rex and found only in the low parent (Rex) and the lowest F_2 bulk, while it was absent in the high parent (Wiley) and the highest F_2 bulk. Moreover, three markers with fragment sizes of 726 (A01 primer), 369 and 208 bp (B08 primer) were detected in the two crosses and found only in the low parents (Roma and Rex) and the lowest F_2 bulk for each cross, while they were absent in the high parent (Wiley) and the highest F_2 bulk for each cross.

Our results are in a harmony with the findings of Abdel-Tawab *et al*. (2004) who used 19 pairs of specific primers to detect the co-segregation of pre-mapped SSR markers with total biomass (TBM) trait in sorghum. Moreover, Abdel-Bary *et al*. (2005) detected three positive and five negative RAPD markers using eight primers as well as one positive SSR marker using five primers for salt tolerance in maize. In addition, Rashed *et al*. (2006) developed five positive and three negative RAPD markers using nine primers as well as two

positive and three negative SSR markers using seven primers for salt tolerance in sorghum. Also, Atta *et al*. (2006) elucidated seven positive and one negative RAPD markers using nine primers as well as three positive and two negative ISSR markers using six primers for iron deficiencey tolerance in maize. Furthermore, Fahmy *et al*. (2007) determined four positive and one negative RAPD markers using eight primers as well as three positive and one negative ISSR markers using elven primers for drought tolerance in rice. Finally, Younis *et al*. (2007) evaluated four positive and ten negative RAPD markers using ten primers as well as six positive and nine negative ISSR markers using ten primers for salt tolerance in grain sorghum.

Marker-assisted selection would enable the molecular breeders to detect the promising lines with more confidence in their merits as line selection based on genetic rather than phenotypic basis. Moreover, this process is fast, reliable, cost effictive and reducing the reqired time for sorghum breeding programs in Egypt.

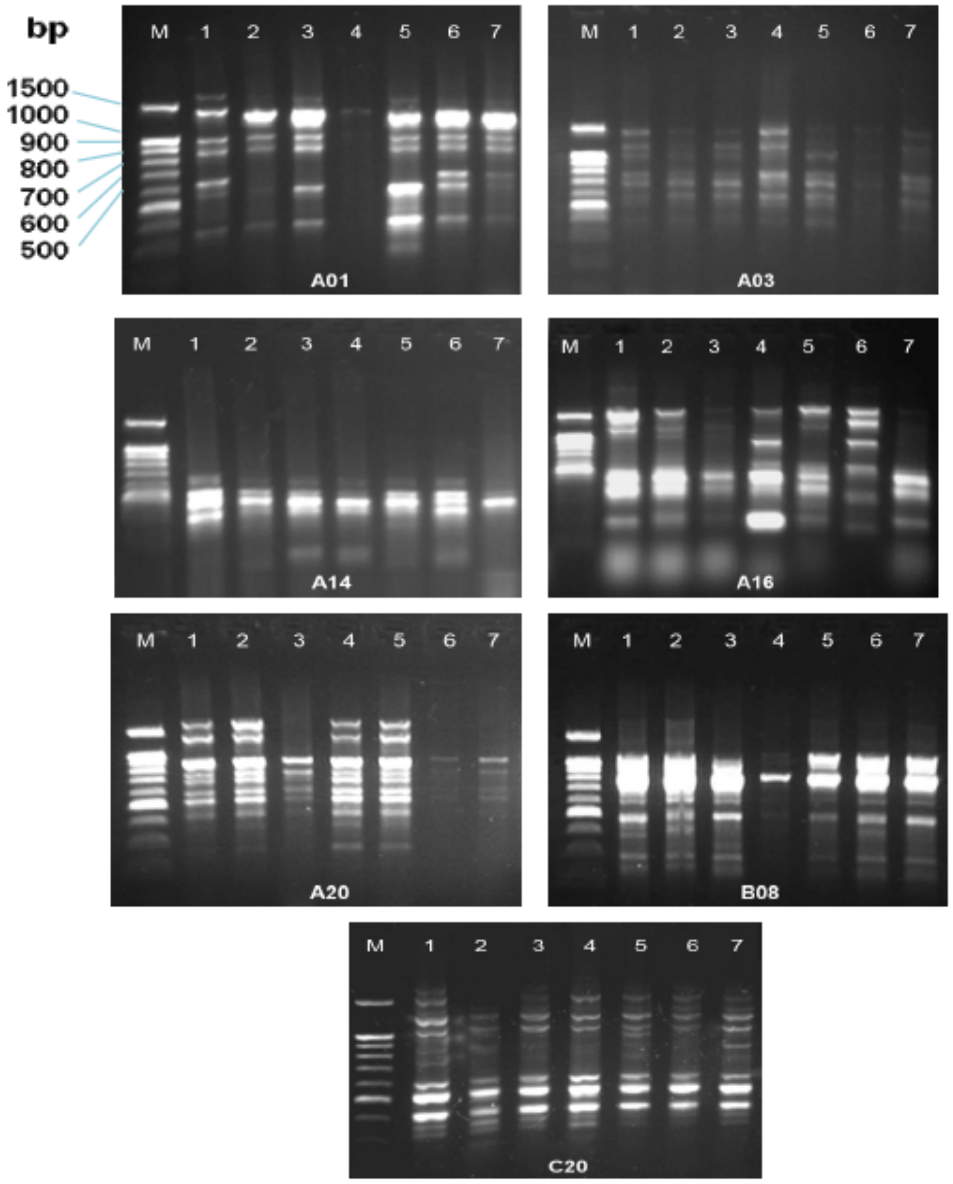

Figure (1): RAPD – PCR fragments using seven primers for Roma (1), Wiley (4) and Rex (7) parents, the highest (2) and the lowest (3) F_2 groups of the Roma x Wiley cross and the highest (5) and the lowest (6) F_2 groups of the Wiley x Rex cross.

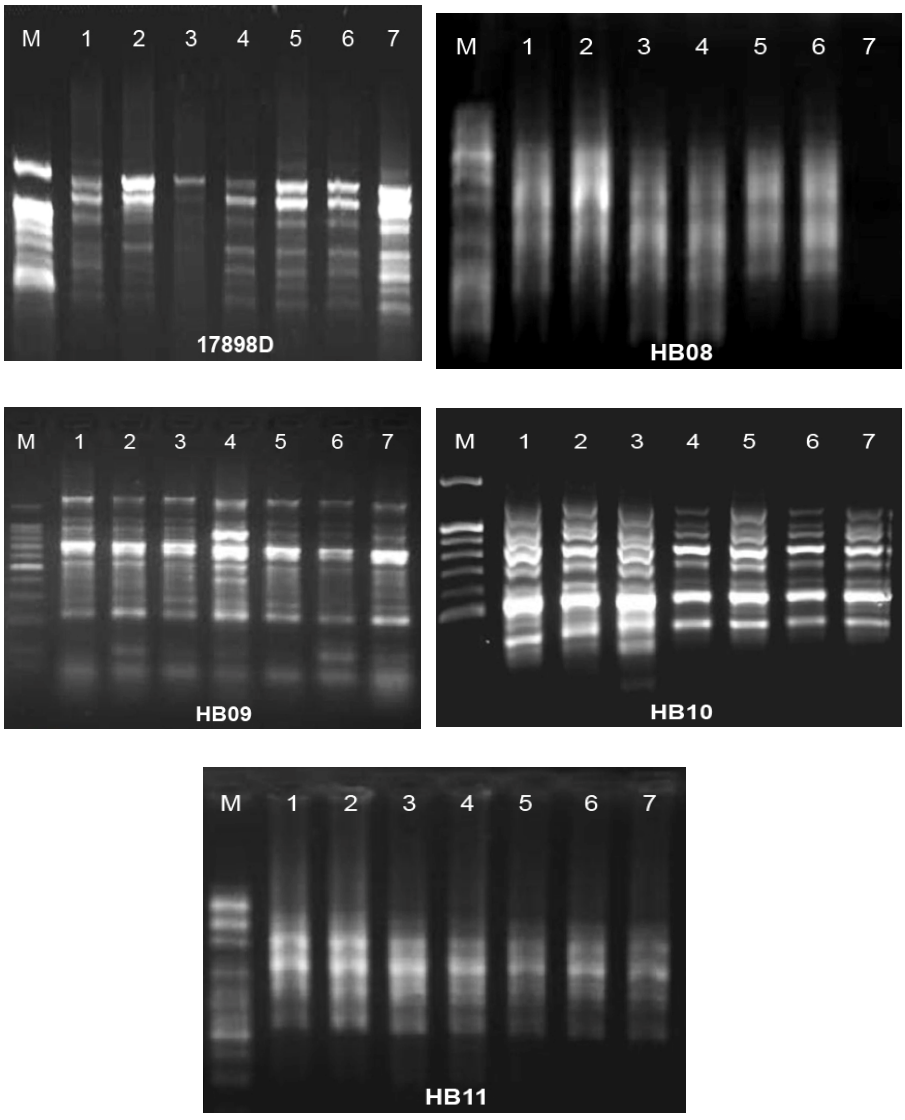

Figure (2): ISSR – PCR fragments using five primers for Roma (1), Wiley (4) and Rex (7) parents, the highest (2) and the lowest (3) F_2 groups of the Roma x Wiley cross and the highest (5) and the lowest (6) F_2 groups of the Wiley x Rex cross.

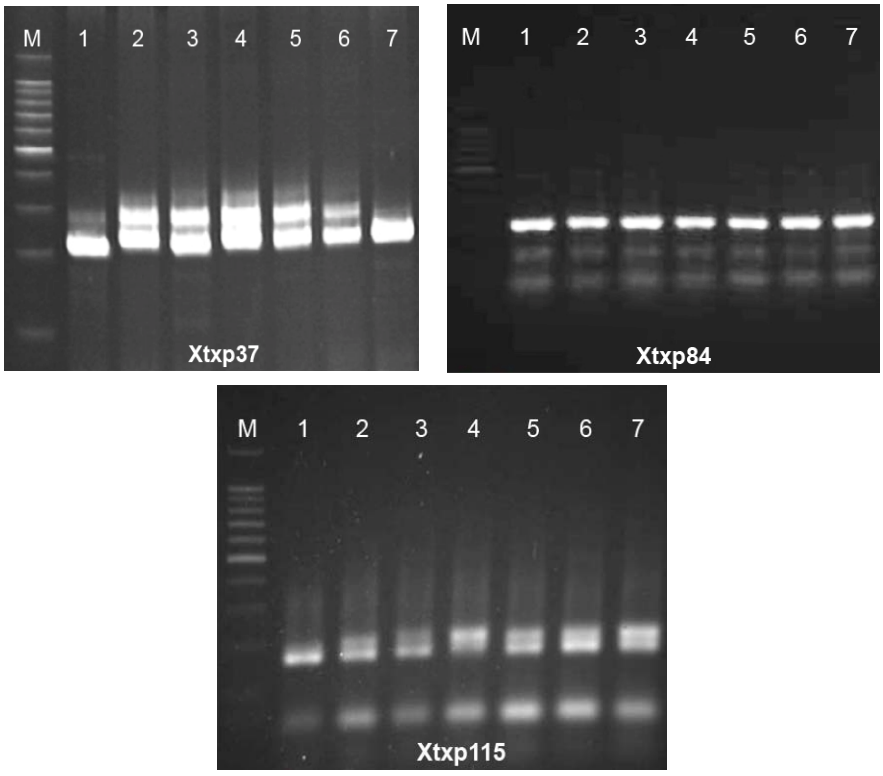

Figure (3): SSR – PCR fragments using three primers for Roma (1), Wiley (4) and Rex (7) parents, the highest (2) and the lowest (3) F_2 groups of the Roma x Wiley cross and the highest (5) and the lowest (6) F_2 groups of the Wiley x Rex cross.

Table (5): RAPD fragments of five RAPD primers with the three parents and DNA bulks of the highest and the lowest F2 groups from each cross (H=high, L= low, N=negative and P= positive).

Primer code	Polymorphic Band no.	Fragment Size (bp)	Roma (L)	Roma x Wiley (H)	Roma x Wiley (L)	Wiley (H)	Wiley x Rex (H)	Wiley x Rex (L)	Rex (L)	Marker type
A01	1	1876	1	1	1	0	1	1	1	----
	3	1402	0	1	1	0	1	1	1	----
	6	726	1	0	1	0	0	1	1	N[Wiley x (Roma & Rex)]
	7	630	1	1	1	0	1	1	1	-----
	8	496	0	0	0	0	1	1	0	----
	9	425	1	1	1	0	1	1	1	-----
	10	318	0	0	0	0	1	0	0	-----
A03	5	632	0	0	0	1	1	0	1	----
	6	626	1	1	1	0	0	0	0	-----
	9	385	1	1	1	1	1	0	0	P (Wiley x Rex)
A16	1	2599	1	0	0	0	0	0	0	----
	2	1789	1	1	1	1	1	1	0	----
	3	1436	1	0	0	0	1	1	0	----
	4	1250	0	0	1	0	0	0	0	----
	5	1140	1	1	0	0	0	0	0	----
	6	1059	0	1	0	1	1	0	0	P [Wiley x (Roma & Rex)]
	7	973	0	0	0	1	0	1	0	----
	8	620	0	0	0	1	1	1	0	----
	9	450	1	1	0	1	1	0	1	----
	10	394	1	1	1	1	1	0	1	----
	11	218	0	0	0	1	1	0	1	----
	12	198	0	1	0	0	0	0	0	----
	13	186	1	0	0	0	0	1	0	----
A20	1	1538	0	1	0	1	1	0	0	P [Wiley x (Roma & Rex)]
	2	1293	0	1	0	1	1	0	0	P [Wiley x (Roma & Rex)]
	3	435	1	1	1	1	1	0	0	P (Wiley x Rex)
	4	372	0	1	0	1	1	0	0	P [Wiley x (Roma & Rex)]
	5	266	0	1	0	1	1	0	0	P [Wiley x (Roma & Rex)]
B08	1	1664	1	1	0	0	1	1	1	----
	2	1298	1	1	0	0	1	1	1	----
	3	1048	0	0	0	1	0	0	0	----
	4	872	1	1	1	0	1	1	1	----
	5	369	1	0	1	0	0	1	1	N[Wiley x (Roma & Rex)]
	6	347	1	0	0	0	0	0	0	----
	7	288	1	1	1	0	0	1	1	N (Wiley x Rex)
	8	208	1	0	1	0	0	1	1	N[Wiley x (Roma & Rex)]

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تحديد بعض الكشافات الجزيئية التى لها علاقة بمحتوى السكر فى الذرة الرفيعة السكرية

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تهدف الدراسة الحالية إلى تقديم ثلاث سلالات من الذرة الرفيعة السكرية لمحتواها من السكر فى الحقل. وقد تم إختيار هذه السلالات على أساس أن السلالة Wiley تعتبر عالية فى محتواها من السكر فى حين أن السلالتين Rex و Roma منخفضتين فى محتواهما من السكر والذي تم التهجين بينهم للحصول على نباتات الجيل الأول والتى بالتالى تركت للتلقيح الذاتى للحصول على نباتات الجيل الثانى لكل هجين من الهجينين Roma X Wiley, Wiley X Rex. وقد تم عمل تجربة حقلية لاختبار الثلاث سلالات والهجينين الناتجين منهما لمحتواهم من السكر بقياس صفتين مرتبطين بمحتوى السكر مثل نسبة السكروز ونسبة المواد الصلبة الذائبة علاوة على صفةالأيام اللازمة للوصول النسبة 50% من heading. تم عمل تجميع للـ DNA المستخلص من المجموعتين الأعلى والأدنى من محتوى السكر لنبات الجيل الثانى لكل هجين وكذلك الثلاثة آباء للإختبارات الوراثية الجزيئية بإستخدام سبعة بادئات لتقنية الـ RAPD وخمسة بادئات لتقنية الـ ISSR وثلاثة بادئات لتقنية الـ SSR لإستنباط كشافات وراثية جزيئية لمحتوى السكر فى الذرة الرفيعة السكرية. ولم يظهر أى تباين للحزم لتقنية ال SSR بينما ظهر فى التقنيتين الأخرتين والذى ظهر منهم فقط خمسة بادئات لتقنية الـ RAPD كشافات جزيئية. وقد تم تحديد سبعة حزم موجبة وأربعة حزم سالبة لصفة محتوى السكر فى الذرة الرفيعة.